A

Practitioner's Docket No. 98 WL 1 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of

Inventor(s): James C. Costin

WARNING: *37 C.F.R. § 1.41(a)(1) points out:*

"(a) A patent is applied for in the name or names of the actual inventor or inventors.

"(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors."

For (title):

METHOD AND COMPOSITIONS FOR THE PREVENTION OF THE DEVELOPMENT OF ANTIBIOTIC DRUG RESISTANCE IN BACTERIA AND THE PREVENTION OF BACTERIA-TO-BACTERIA TRANSFER OF ANTIBIOTIC DRUG RESISTANCE

CERTIFICATION UNDER 37 C.F.R. 1.10*
(Express Mail label number is mandatory.)
(Express Mail certification is optional.)

I hereby certify that this New Application Transmittal and the documents referred to as attached therein are being deposited with the United States Postal Service on this date September 11, 1998, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number TB725860234US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Kevin B. Clarke
(type or print name of person mailing paper)

Kevin B. Clarke
Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

WARNING: *Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

1. Type of Application

This new application is for a(n)

(check one applicable item below)

☒ Original (nonprovisional)

☐ Design

☐ Plant

WARNING: ***Do not*** *use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application.*

WARNING: ***Do not*** *use this transmittal for the filing of a provisional application.*

NOTE: If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

☐ Divisional.

☐ Continuation.

☐ Continuation-in-part (C-I-P).

2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

NOTE: A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. 112. Each prior application must also be:

(i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America; or

(ii) Complete as set forth in § 1.51(b); or

(iii) Entitled to a filing date as set forth in § 1.53(b) or § 1.53(d) and include the basic filing fee set forth in § 1.16; or

(iv) Entitled to a filing date as set forth in § 1.53(b) and have paid therein the processing and retention fee set forth in § 1.21(l) within the time period set forth in § 1.53(f).

37 C.F.R. § 1.78(a)(1).

NOTE: If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

WARNING: *When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application must be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).*

☐ The new application being transmitted claims the benefit of prior U.S. application(s). Enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed

A. Required for filing date under 37 C.F.R. § 1.53(b) (Regular) or 37 C.F.R. § 1.153 (Design) Application

__13__ Pages of specification

__1__ Pages of claims

__0__ Sheets of drawing

☐ formal

☐ informal

B. Other Papers Enclosed

__1__ Pages of Abstract

__0__ Other

WARNING: ***DO NOT*** *submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. For comments on proposed then-new 37 CFR 1.84, see Notice of March 9, 1988 (1990 O.G. 57-62).*

NOTE: "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8 inch) down from the top of the page . . ." 37 C.F.R. 1.84(c)).

(complete the following, if applicable)

☐ The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)." 37 C.F.R. 1.84(b).

4. Additional papers enclosed

☐ Preliminary Amendment

☐ Information Disclosure Statement (37 C.F.R. 1.98)

☐ Form PTO–1449 (PTO/SB/08A and 08B)

☐ Citations

☐ Declaration of Biological Deposit

☐ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.

☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative

☐ Special Comments

☐ Other

5. Declaration or oath

NOTE: A newly executed declaration is not required in a continuation or divisional application provided that the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47, then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 C.F.R. §§ 1.63(d).

☒ Enclosed

Executed by

(check **all** *applicable boxes)*

☒ inventor(s).

☐ legal representative of inventor(s). 37 CFR 1.42 or 1.43.

☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.

☐ This is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached. See item 13 below for fee.

☐ Not Enclosed.

NOTE: Where the filing is a completion in the U.S. of an International Application or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.

☐ Application is made by a person authorized under 37 C.F.R. 1.41(c) on behalf of *all* the above named inventor(s).

(The declaration or oath, along with the surcharge required by 37 CFR 1.16(e) can be filed subsequently).

NOTE: It is important that all the correct inventor(s) are named for filing under 37 CFR 1.41(c) and 1.53(b).

☐ Showing that the filing is authorized. *(not required unless called into question. 37 CFR 1.41(d))*

6. Inventorship Statement

WARNING: *If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.*

The inventorship for all the claims in this application are:

☒ The same.

or

☐ Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,

☐ is submitted.

☐ will be submitted.

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of $130.00 required by 37 CFR 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 CFR 1.52(d).

☒ English

☐ Non-English

☐ The attached translation includes a statement that the translation is accurate. 37 C.F.R. 1.52(d).

8. Assignment

☒ An assignment of the invention to Carter-Wallace, Inc.

☒ is attached. A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

☐ will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters-one for the application and one for the assignment." Notice of May 4, 1990 (1114 O.G. 77-78).

***WARNING:** A newly executed "CERTIFICATE UNDER 37 CFR 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O.G. 62-64.*

9. Certified Copy

Certified copy(ies) of application(s)

Country	Appln. No.	Filed
Country	Appln. No.	Filed
Country	Appln. No.	Filed

from which priority is claimed

☐ is (are) attached.

☐ will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 CFR 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application, then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 C.F.R. 1.16)

A. ☒ Regular application

CLAIMS AS FILED					
Number filed		Number Extra		Rate	Basic Fee 37 C.F.R. 1.16(a) $790.00
Total Claims (37 CFR 1.16(c))	4 − 20 =	0	×	$ 22.00	0
Independent Claims (37 CFR 1.16(b))	3 − 3 =	0	×	$ 82.00	0
Multiple dependent claim(s), if any (37 CFR 1.16(d))	1		+	$270.00	$270.00

☐ Amendment cancelling extra claims is enclosed.

☐ Amendment deleting multiple-dependencies is enclosed.

☐ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 CFR 1.16(d).

Filing Fee Calculation $1,060.00

B. ☐ Design application
($330.00—37 CFR 1.16(f))

Filing Fee Calculation $_____

C. ☐ Plant application
($540.00—37 CFR 1.16(g))

Filing fee calculation $_____

11. Small Entity Statement(s)

☐ Statement(s) that this is a filing by a small entity under 37 CFR 1.9 and 1.27 is (are) attached.

WARNING: *"Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under § 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent or includes a copy of the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this section." 37 C.F.R. § 1.28(a)(2).*

(complete the following, if applicable)

☐ Status as a small entity was claimed in prior application

_____ / _____, filed on _____, from which benefit is being claimed for this application under:

35 U.S.C. ☐ 119(e),
☐ 120,
☐ 121,
☐ 365(c),

and which status as a small entity is still proper and desired.

☐ A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of **A**, **B** or **C** above)

$_____

NOTE: Any excess of the full fee paid will be refunded if small entitiy status is established and a refund request are filed within 2 months of the date of timely payment of a full fee. The two-month period is not extendable under § 1.136. 37 CFR 1.28(a).

12. Request for International-Type Search (37 C.F.R. 1.104(d))

(complete, if applicable)

☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made at This Time

☐ Not Enclosed

☐ No filing fee is to be paid at this time.
(This and the surcharge required by 37 C.F.R. 1.16(e) can be paid subsequently.)

☒ Enclosed

☒ Filing fee	$ 1,060.00
☒ Recording assignment ($40.00; 37 C.F.R. 1.21(h)) (See attached "COVER SHEET FOR ASSIGNMENT ACCOMPANYING NEW APPLICATION".)	$ 40.00
☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached ($130.00; 37 C.F.R. 1.47 and 1.17(l))	$ _____
☐ For processing an application with a specification in a non-English language ($130.00; 37 C.F.R. 1.52(d) and 1.17(k))	$ _____
☐ Processing and retention fee ($130.00; 37 C.F.R. 1.53(d) and 1.21(l))	$ _____
☐ Fee for international-type search report ($40.00; 37 C.F.R. 1.21(e))	$ _____

NOTE: 37 CFR 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 CFR 1.53(f) and this, as well as the changes to 37 CFR 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of § 1.21(l) must be paid, within 1 year from notification under § 53(f).

Total fees enclosed $ 1,100.00

14. Method of Payment of Fees

☐ Check in the amount of $_____

☒ Charge Account No. 03-0935 in the amount of $ 1,100.00.

A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 CFR 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: *If no fees are to be paid on filing, the following items should not be completed.*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.*

☒ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. 03-0935:

☒☒ 37 C.F.R. 1.16(a), (f) or (g) (filing fees)

☒☒ 37 C.F.R. 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

☐ 37 C.F.R. 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

☐ 37 C.F.R. §§ 1.17(a)(1)-(5) (extension fees pursuant to § 1.136(a)).

☐ 37 C.F.R. 1.17 (application processing fees)

NOTE: ". . .A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

☐ 37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b).

NOTE: 37 CFR 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . . issue fee." From the wording of 37 CFR 1.28(b), (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions as to Overpayment

NOTE: ". . . Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

☒ Credit Account No. 03-0935

☐ Refund

SIGNATURE OF PRACTITIONER

Reg. No. 22,647

Kevin B. Clarke, Esq.
(type or print name of attorney)

Tel. No. (212) 339-5207

Carter-Wallace, Inc.
P.O. Address

Customer No. 004370

1345 Avenue of the Americas
New York, NY 10105

☐ **Incorporation by reference of added pages**

(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

☐ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added _____

☐ Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added _____

☐ Plus added pages deleting names of inventor(s) named in prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.

Number of pages added _____

☒ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added 6

☐ **Statement Where No Further Pages Added**

(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)

☐ This transmittal ends with this page.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES PATENT APPLICATION

FOR: METHOD AND COMPOSITIONS FOR THE PREVENTION OF THE DEVELOPMENT OF ANTIBIOTIC DRUG RESISTANCE IN BACTERIA AND THE PREVENTION OF BACTERIA TO BACTERIA TRANSFER OF ANTIBIOTIC DRUG RESISTANCE

BY: JAMES C. COSTIN, M.D.

ABSTRACT

The use of 4,4-methylenebis (tetrahydro-1,2,4-thiadiazine-1,2-dioxide) in the prevention and control of the development of antibiotic drug resistance in bacteria and in the prevention of bacteria-to-bacteria transfer of genes capable of resisting antibiotics is disclosed.

This application is a continuation-in-part of United States Provisional Application Number 60/058,497 filed September 11, 1997.

This invention relates to a method and compositions for the treatment of bacterial infection which reduces or eliminates the ability of bacteria to acquire resistance to antibiotic drug treatment. Moreover, this invention relates to a method and compositions for the reduction or elimination of bacteria-to-bacteria transfer of antibiotic drug resistance through genetic or other means.

Specifically, the present invention relates to the use of 4,4'-methylenebis(tetrahydro-1,2,4-thiadiazine-1,2-dioxide) known generically as taurolidine to treat antibiotic drug (e.g. gentamicin, vancomycin) resistant bacterial infections, nosocomial infections and/or eradication of these organisms from an individual acting as a "carrier" for these organisms.

Further, the present invention relates to the use of taurolidine to prevent the development of antibiotic drug resistance in bacterial and nosocomial infections.

Lastly, the present invention relates to the use of taurolidine to prevent the bacteria-to-bacteria transfer of antibiotic drug resistance through genetic or other means.

The development of antimicrobial agents has, without question, been one of the crowning achievements of medical science in the latter half of the twentieth century. However, despite the fact that dozens of classes of compounds have been developed, microorganisms, especially bacteria, have developed resistance to virtually every agent which has been subjected to extensive clinical use. As we approach the end of the

twentieth century, there has been a precipitous decline in the development of new antimicrobial agents. There are several reasons for this including the fact that most of the easy targets that allow selective toxicity for antimicrobial agents have been discovered and the fact that it is increasingly expensive to bring a new antimicrobial agent from discovery to the marketplace. There is, however, a major need for discovery of novel classes of antimicrobial agents to which multi-resistant bacteria remain susceptible. Taurolin is a novel new antimicrobial agent. It has a formulation which comprises taurolidine (4~- methylene bis (tetrahydro-1, 2, 4 thiadiazine 1, 1 dioxide). A derivative of aminosulphonic acid taurineamide, this is a novel bactericidal agent that has a unique spectrum of antimicrobial activity that, in preliminary tests, has included Gram-positive and Gram-negative bacteria and fungi. It has been subjected to early clinical trials and it appears to have useful activity in vivo when administered by intravenous or intraperitoneal routes. This compound also has the ability to neutralize endotoxin in vitro and it also exhibits marked anti-adherence properties in vitro.

Additionally, taurolidine has a low potential for toxicity. Doses of 600 mg/kg over 24 hours are non-toxic in experimental animals. Two percent (2%) solutions at doses of 100 mg/kg have been infused intravenously over 60 min in rabbits. The LD_{50} in rats exceeds 4,000 mg/kg.

The emergence of multiple drug resistant enterococci currently poses an enormous threat, especially to hospitalized patients. Many such isolates are resistant to all currently used antimicrobials, and treatment with investigational agents is sometimes necessary. In addition, it has been shown in the laboratory that vancomycin

resistance genes can be transferred to Staphylococcus aureus in which they are expressed; therefore, there is great concern that this will occur in nature, jeopardizing the most effective current antimicrobial agent for treatment of infections due to methicillin-resistant strains of this species. Clearly, new agents with activities against enterococci and other resistant gram-positive pathogens are needed.

Taurolidine has demonstrated bactericidal activity against a broad range of microorganisms and taurolidine has the additional advantage of acting through mechanisms unlike those described for other currently available antimicrobial chemotherapeutic agents. This compound demonstrates activity in vitro against Enterococcus faecalis at concentrations <2000 μg/ml, with minimum inhibitory concentrations (MIC_{50}s) of 125-250 μg/ml and (MIC_{90}s) of 250-1000 μg/ml. The major metabolite, taurultam, is described as being approximately 50% as potent. Interestingly, taurolidine demonstrates antibacterial activity in vivo despite plasma concentrations which are an order of magnitude below the MIC. Reasons for this major discordance between in vitro and in vivo activities of the agent are not understood, but several factors may contribute. The compound demonstrates endotoxin neutralizing activity, inhibits adherence, is more active at low pH which may prevail at the site of infections or within phagolysosomes, is slightly more active when tested in serum-supplemented media, and inhibits potential bacterial toxins such as staphylococcal coagulase.

The precise mode of action of taurolidine has not been fully elucidated. In simple aqueous solution, taurolidine exists in equilibrium with taurultam and methylol-

donating species. Current consensus suggests hydrolysis of taurolidine in vivo to methylol taurultam and taurultam in equilibrium. Upon liberation of one active N-methylol (hydroxymethyl) group from methylol taurultam, taurultam is further hydrolyzed via methylol taurineamide to taurine. Thus, three active N-methylol groups are liberated per molecule of taurolidine following reaction with bacterial or fungal cell constituents. These methylol groups have a high affinity for, and bind selectively and irreversibly to bacterial cell wall constituents to exert their bactericidal affect. Because of this unique mechanism of action, there is no reason to suspect cross-resistance with standard antimicrobial agents that do not share this mechanism of action.

Taurolidine's general characteristics include acceptable stability in the solid state when stored at ambient conditions, melting with decomposition at approximately 170°C and the following solubility in aqueous solutions and organic solvents.

Water	1% at 20°C
Dilute HCl	soluble
Dilute NaOH	soluble
$CHCl_3$	insoluble
EtOH	sparingly soluble
DMF	1 g in 2 mL/ca.60°C
Acetone	1 g in 120 mL/Boiling
Ethanol	1 g in 130 mL/Boiling
Methanol	1 g in 170 mL/Boiling
Ethyl Acetate	1 g in 200 mL/Boiling

A saturated solution of taurolidine in deionized water has a pH of 7.4. The apparent partition coefficient of taurolidine between octanol and water (buffered at pH 7.2) is approximately 0.13 and would therefore not be predicted to accumulate to any significant extent in fatty tissues.

The synthesis of taurolidine is covered in a number of patents including USA 3,423,408; Switzerland No. 482,713 and United Kingdom No. 1,124,285 and is carried out in five stages:

* Potassium phthalimidoethane sulphonate is prepared from taurinc, phthalic anhydride, glacial acetic acid and potassium acetate;
* Potassium phthalimidoethane sulphonate is then converted to phthalimidoethane sulphonylchloride by chlorination with phosphorous oxychloride;
* Phthalimidoethane sulphonylchloride is reacted with ammonia to form phthalimidoethane sulphonamide;
* Phthalimidoethane sulphonylchloride is reacted with hydrazine hydrate and in the subsequent hydrazinolysis to form taurinamide hydrochloride; and
* Taurolidine is prepared from taurinamide hydrochloride and formaldehyde.

The antimicrobial actions of taurolidine have been described in United States Patent 3,423,408 and elsewhere in the literature. In addition, the following United States Patents describe various uses for and compositions containing taurolidine: U.S. 4,107,305, treatment of endotoxaemia; U.S. 4,337,251, elimination of adhesion formation as a result of surgery; U.S. 4,587,268, resorbable aqueous gels; U.S. 4,604,391, prevention of the occurrence of osteitis or osteomyelitis; U.S. 4,626,536, combatting toxic proteins or peptides in the blood; U.S. 4,772,468, treatment of bone cavities; and U.S. 4,882,149, directed to methods for filling congenital, surgical or

traumatic defects with compositions comprising natural bone mineral having absorbed therein/thereon taurolidine.

Taurolidine has been shown to be safe and well tolerated at systemic doses exceeding 40g/day and cumulative doses up to and exceeding 300g.

The formulations of taurolidine generally utilized are sterile solutions containing 0.5%, 1.0% or 2.0% taurolidine for irrigation/lavage, wound instillation, intravenous or oral administration, primarily for the treatment or prevention of peritonitis, sepsis or osteitis/osteomyelitis. In addition, topical surgical gels containing 2.0% to about 4.0% are utilized for the treatment of osteitis/osteomyelitis.

It has long been the goal of the pharmaceutical industry to produce antibiotic medicinal substances that have the power to kill - or at least to arrest the growth of - many disease causing bacteria such as the streptococci, enterococci and staphylococci.

It has also been observed that the susceptibility of bacteria to various antibiotic medicines can change markedly over time, i.e., the antibiotic gentamicin was widely used for about ten years to treat staphylococcal infection until the bacteria acquired a resistance to gentamicin. The realization that infectious bacteria could become immune to all available antibiotics has raised alarm in the medical community which now cautions doctors that over prescribing antibiotics can hasten the evolution of resistant germs.

Moreover, a study by the Federal Centers for Disease Control in Atlanta, Georgia, has shown that nearly eight percent of all enterococci isolated in hospitals

nationwide were resistant to vancomycin, the antibiotic considered to be the last line of defense against organisms impervious to other drugs. This was more than 20 times the rate of resistance to vancomycin detected only four years earlier.

Of equal concern to the medical community is the ability of a bacteria, once it has acquired an immunity to an antibiotic, to transfer such immunity to other bacterium.

Resistance to antibiotics is developed in bacterium cells in a small circle of DNA known as plasmid which is matter consisting of a double-stranded DNA that is apart from the chromosomes but carries genes for a variety of functions and can replicate itself. The genes are concerned with such functions as resistance to antibiotics. Plasmids are separate from the rest of the bacterium, and they can move quite easily from one bacterium to another. This transferability of plasmids enables resistance genes to spread rapidly even among different species of bacteria. Transfer between bacteria of plasmids is accomplished through the use of F pili which are fine filaments resembling flagellum which are outgrowths from the bacteria cells which normally function to propel the cell, however when the F pili attach to another cell, a bridge is formed which permits the plasmids to spread rapidly from one cell to another.

Antibiotics generally work by interfering with the construction of the bacterial cell wall. In the case of vancomycin-resistance, its action can be thwarted by the bacteria modifying the building blocks of their cell walls by substituting a molecule of lactic acid for one of analine. The most common plasmid that confers resistance to vancomycin has a package of nine genes that set in motion this modification. A first gene enables the bacterium to manufacture lactic acid, a second gene codes for an enzyme that can

cleve the analine from the cell wall building block, the product of a third gene puts lactic acid in the analines place. Two more genes control the previous three ensuring that they are activated only in the presence of vancomycin. The remaining genes are involved in helping the resistance package mobilized itself in different ways.

It has now been found that taurolidine in addition to its known antimicrobial, antitoxin, antibacterial and antifungal properties destroys antibiotic resistant strains of staphylococci, enterococci and other bacterial and nosocomial infections, prevents the development of antibiotic resistance in staphylococci, enterococci and other bacterial and nosocomial infections and prevents the transfer of bacteria-to-bacteria drug resistance through genetic or other means.

Microbial adherence to mucosal epithelial cells is recognized as a significant step in the successful colonization of the intestinal, respiratory and genito-urinary tracts in the early stages of infection. The attachment and agglomeration of organisms is important, both in the pathogenesis of infection and in limiting the response to antibiotic treatment.

Taurolidine has been found to significantly reduce the adherence of buccal and vaginal isolates of candida albicans blastospores and urine isolates of escherichia coli and staphylococcus saprophyticus to epithelial cells. Light microscopy and radio-isotopic counting methods were used to quantify the adherence of the microorganisms to either uropithelial or buccal epithelial cells.

Treatment of either epithelial cells or microorganisms with taurolidine resulted in reduced adherence of microorganisms.

Using a thirty minute contact time, a range of taurolidine concentrations on the order of 0.05% to about 2.0% w/v were examined for antiadherence activity. Significant decreases in candida blastospore adherence were observed at concentrations of less than 0.1% w/v taurolidine. Maximum reductions in adherence, on the order of about 65% of control were observed when concentrations of taurolidine greater than about 0.5% w/v. Increasing the taurolidine concentration beyond this level did not produce a concomitant increase in antiadherence activity. Conversely, dilution of taurolidine concentration may proceed to a considerable extent before its capacity for antiadherence is lost.

The foregoing demonstrates that taurolidine exerts an antiadherence activity via a chemical modification of outer surface structures such as fimbriae causing agglutination or disappearance of the structures. The effect of taurolidine on these structures which contribute to the initiation of infection and in determining the pathogenicity of the organism is clear evidence of one aspect of taurolidine's mechanism of action in preventing infection or reducing its severity.

As noted above, taurolidine's mechanism of action unlike that of known antibiotics is based on a chemical reaction. While not being bound by any theory, during the metabolism of taurolidine to taurinamide and ultimately taurine and water, methylol groups are liberated which chemically react with the mureins in the bacterial cell walls this results in the denaturing of the complex polysaccharide and liposaccharide components of the bacterial cell wall as well as changing the double stranded DNA of the plasmid to a denatured or single stranded DNA.

Example 1, which follows, demonstrates taurolidine's activity against vancomycin-resistant enterococci.

EXAMPLE 1

Twelve clinical isolates of vancomycin-resistant enterococci, each with a vancomycin minimum inhibatory concentration of $\geq$ 128 μg/ml were challenged in vitro with taurolidine by pulsed-field gel electrophoresis. Ten of the vancomycin-resistant enterococci strains were genotypically distinct and two of the strains were genetically-related.

Additionally, taurolidine activity was tested against vancomycin-sensitive enterococci-E.Faecalis (American Type Culture Collection #29212) and E.Faecium (American Type Culture Collection #35667). Susceptibility testing was performed using broth dilution methods and geometric means were used to determine the minimum inhibitory concentration and minimum bactericidal concentration values.

For the twelve vancomycin-resistant enterococci strains:

MIC_{50} = 400 μg/ml
MIC_{90} = 500 μg/ml

MBC_{50} = 1010 μg/ml
MBC_{90} = 1500 μg/ml

For the American Type Culture Collection strains of E.Faecalis and E.Faecium:

MIC = 310 μg/ml and 360 μg/ml
MBC = 960 μg/ml and 980 μg/ml, respectively.

For two vancomycin-resistant strains, the minimum inhibitory concentration:

at pH 5 = 100 μg/ml
at pH 6 = 200 μg/ml

at pH 6.5 = 500 μg/ml
at pH 7 = 500 μg/ml

For five vancomycin-resistant strains, the minimum inhibitory concentrations with no serum added equals 100-400 μg/ml and the minimum bactericidal concentration was 400-800 μg/ml.

In 95% rabbit serum, the minimum inhibitory concentrations are 200-400 μg/ml and the minimum bactericidal concentrations are 400-600 μg/ml.

The foregoing data demonstrates that taurolidine has inhibitory and cidal activity against vancomycin-resistant clinical isolates. Although the minimum inhibitory and minimum bactericidal concentrations at physiologic pH are above achievable serum levels, the data demonstrates that an enteral preparation of taurolidine will have an effect on vancomycin-resistant enterococci gastrointestinal carriage.

Example 2, which follows, demonstrates the activity of taurolidine against vancomycin-intermediate susceptibility staphylococcus aureus and methicillin-resistant staphylococcus aureus.

EXAMPLE 2

The in vitro activity of taurolidine was tested against two clinical isolates of vancomycin-intermediate susceptible staphylococcus aureus identified as Mu3 and Mu50 and five clinical methicillin-resistant staphylococcus aureus isolates. Susceptibility testing was performed using broth dilution methods and geometric means were used to determine the MIC_{50} and MIC_{90} values.

For the Mu3 vancomycin-intermediate susceptible staphylococcus aureus isolate:

MIC = 500 μg/ml
MBC = 1100 μg/ml

and for the Mu50 isolate, the

MIC = 500 μg/ml
MBC = 840 μg/ml

For the methicillin-resistant staphylococcus aureus isolates:

MIC_{50} = 550 μg/ml
MIC_{90} = 575 μg/ml

and

MBC_{50} = 1025 μg/ml
MBC_{90} = 1150 μg/ml

For the Mu3 vancomycin-intermediate susceptible staphylococcus aureus isolate:

MIC at pH 5 = 200 μg/ml
MIC at pH 6 = 500 μg/ml and
MIC at pH 7 = 750 μg/ml

For the Mu50 vancomycin-intermediate susceptible staphylococcus aureus isolate:

MIC at pH 5 = 50 μg/ml
MIC at pH 6 = 200-500 μg/ml and
MIC at pH 7 = 500 μg/ml

For two methicillin-resistant staphylococcus aureus isolates, the minimum inhibitory concentration with no serum added equals 500-1000 μg/ml and the minimum bactericidal concentration equals 1000-2000 μg/ml. After adding 95% rabbit serum, the

minimum inhibitory concentration equals 500 μg/ml and the minimum bactericidal concentration equals 500-1000 μg/ml.

The foregoing data demonstrates that taurolidine has inhibitory and cidal activity against vancomycin-intermediate susceptibility staphylococcus aureus isolates and against methicillin-resistant staphylococcus aureus isolates.

The addition of serum did not have any significant effect on the activity of taurolidine. The minimum inhibitory concentration and the minimum bactericidal concentration at physiologic pH are above achievable serum levels, however, the data clearly demonstrates that taurolidine does have an effect on nasopharyngeal vancomycin-intermediate susceptibility staphylococcus aureus and methicillin-resistant staphylococcus aureus carriage if applied topically.

Various modifications may be made in the invention described herein. Accordingly, the scope of the invention is defined in the following claims wherein:

WHAT IS CLAIMED IS:

1. A method for the prevention of the development of antibiotic drug resistance in bacteria which comprises administering to a human or other warm blooded animal, infected with bacteria, the compound 4,4-methylenebis(tetrahydro-1,2,4-thiadiazine-1,2-dioxide).

2 A method for the prevention of bacteria to bacteria transfer of plasmid materials containing genes capable of resisting antibiotics which comprises administering to a human or other warm blooded animal harboring bacteria containing such plasmids the compound 4,4-methylenebis (tetrahydro-1,2,4-thiadiazine-1,2-dioxide).

3. The method of claim 1 wherein said 4,4-methylenebis (tetrahydro-1,2,4-thiadiazine-1,2-dioxide) is combined with an additional antibiotic or antibiotics.

4. The methods of claims 1-3 wherein said compounds are administered orally.

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION, OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is for an original application.

INVENTORSHIP IDENTIFICATION

My residence, post office address and citizenship are as stated below, next to my name. I believe that I am the original, first and sole inventor *(if only one name is listed below)* or an original, first and joint inventor *(if plural names are listed below)* of the subject matter that is claimed, and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

METHOD AND COMPOSITIONS FOR THE PREVENTION OF THE DEVELOPMENT OF ANTIBIOTIC DRUG RESISTANCE IN BACTERIA AND THE PREVENTION OF BACTERIA-TO-BACTERIA TRANSFER OF ANTIBIOTIC DRUG RESISTANCE

SPECIFICATION IDENTIFICATION

The specification is attached hereto.

ACKNOWLEDGMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, § 1.56.

POWER OF ATTORNEY

I hereby appoint the following practitioner(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Kevin B. Clarke, Esq. Registration Number 22,647

I hereby appoint the practitioner(s) associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

SEND CORRESPONDENCE TO

Kevin B. Clarke, Esq.
Carter-Wallace, Inc.
1345 Avenue of the Americas
New York, NY 10105 U.S.A.

Customer Number 004370

DIRECT TELEPHONE CALLS TO:

Kevin B. Clarke, Esq.
(212) 339-5207

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

James C. Costin
Inventor's signature _____
Date 9-8-98 **Country of Citizenship** U.S.A.
Residence NEW YORK, NY
Post Office Address 1106 Hunt Seat Drive
Lower Gwynedd, PA 19002 U.S.A.